



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A01N 37/10, 43/36	A1	(11) International Publication Number: WO 00/05954 (43) International Publication Date: 10 February 2000 (10.02.00)
(21) International Application Number: PCT/US99/16365 (22) International Filing Date: 20 July 1999 (20.07.99) (30) Priority Data: 09/126,202 30 July 1998 (30.07.98) US (71) Applicant: STOLLER ENTERPRISES, INC. [US/US]; Suite 200, 8580 Katy Freeway, Houston, TX 77024 (US). (72) Inventor: DEAN, Frank, W.; 25414 Sugar Valley, Spring, TX 77373 (US). (74) Agent: BROOKHART, Walter, R.; Browning Bushman, Suite 1800, 5718 Westheimer Rd., Houston, TX 77057 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: TREATMENT OF PLANTS WITH SALICYLIC ACID AND ORGANIC AMINES (57) Abstract The present invention is directed to processes for improving plant resistance to disease without inhibiting plant growth. In the processes of the present invention a plant is treated with an ortho-substituted benzoic acid, preferably salicylic acid, and a nitrogen-containing compound selected from the group consisting of the organic amines and compounds which will metabolize to an organic amine. Preferred are the polyamines, tertiary amines, propylamines and organic compounds which will metabolize to produce a propylamine. Optionally, the plants are simultaneously treated with a chelated micronutrient metal preferably selected from the group consisting of the alkaline earth metals, the transition metals and boron. Finally, the plants are optionally treated with an ethylene-inducing compound, preferably indole-3-butyric acid. Preferred method of application is to the foliage of the plant by way of a solution in a suitable carrier medium.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TREATMENT OF PLANTS WITH SALICYLIC ACID AND ORGANIC AMINES

Background of the Invention

I. Field of the Invention

The present invention generally relates to methods for improving disease resistance in plants without adversely affecting the plant growth. More specifically, the present invention is directed to methods for achieving such results by treating the plant tissue with an ortho- substituted benzoic acid and an organic amine.

II. Description of the Background

Agricultural pesticides are used to control unwanted fungal, viral, and bacterial populations. These compounds have allowed the producer to manage disease pressure. Although, these traditional chemical applications have been valuable in the past it is unlikely that the producer will be able to use them at the same rate into the future. Therefore, improved methods for controlling disease by stimulating the plant's natural processes are desirable.

For some years it has been known that plants have the ability to produce pathogenesis proteins. Once these proteins are expressed plants may have the capacity to protect themselves from pathogenic insults. The efforts of individuals, chemical producers, and academic institutions are now concentrated on producing genetically manipulated species that either repress or express these enzymes or their perceived precursors. Innumerable compounds have been documented as inducers of the pathogenesis proteins. However, because the compounds applied separately induce a given set or class of enzymes they have not been found to be efficacious in preventing infection in field hosts.

An inoculation with an array of elicitors would induce the expression of recessive enzymes of two or more classes. These enzymes would enable the treated plant to defend itself against pathogens.

Plants are continually under attack from pathogenic microorganisms. The pathogenic insults from the exposure to fungal, viral, and bacterial assaults are frequently confined to the area of attack. These insults often cause lesions as the cell dies. The cell death is part of a hypersensitive reaction (HR) to the pathogen. This reaction acts as an inoculant for the lesion forming pathogen,

-2-

and also, as an inoculant for unrelated pathogens. This inoculation provides a systemic acquired resistance (SAR). It is now well established that some phenolic compounds can induce these pathogenesis related proteins in the absence of the pathogenic organisms. These compounds include cinnamic, benzoic, coumaric, and salicylic acids and salts. It has been found that exposing a plant to such phenolic compounds prior to the attack of the pathogen can minimize the effects. The pathogenesis proteins are chitinase enzymes. Three classes of chitinase have been identified as follows:

- Class I - catalytic activity, cystine rich (protein spacer between amino and carboxyl ends) – ethylene induced.
- Class II - catalytic activity (all are part of the protein spacer) – SAR induced.
- Class III - catalyst activity – SAR induced.

It is assumed the expression of all three classes of Chitinases simultaneously would provide greater protection than the expression of only a single protein. Ethylene, a naturally occurring plant hormone, often used to induce certain events in the plant life cycle, has been shown to induce expression of pathogenesis proteins. Chitinase has been found in ethylene treated leaves. It has been suggested that these basic enzymes serve to protect plants against potential pathogens. Ethylene, a gas, is expressed throughout the plant kingdom in response to drought, flooding, frost, and physical injury. The plant hormone has also been reported to be expressed with chemical applications. Auxins have been shown to induce expression of ethylene at very low concentrations when applied. They are also useful for the inducement of chitinase. Auxins have also been shown to induce a class of pathogenesis proteins. Sugars have also been shown to induce ethylene biosynthesis.

Two classes of Chitinases have been identified that are associated with the onset of systemic acquired resistance. While transition metals are needed for nutritional purposes, foliar application of manganese has been shown to induce a class of pathogen related (PR) proteins. Zinc, as a nutrient supplied

-3-

above optimal levels, has been shown to suppress *Spongossora subterranea* in watercress. High zinc levels effectively suppress watercress chlorotic leaf spot virus through control of its vector, the fungus.

Systemic resistance is typically characterized by a reduction in lesion size.

- 5 The lesion response is a programmed cell death to isolate the pathogen and prevent further infection. When the pathogenesis related proteins are expressed prior to disease inoculation the pathogens can be isolated within the lesion in a shorter time period. It has been postulated that those substances that induce pathogenesis related proteins would *prime* the plant for disease resistance, if
10 applied prior to the insults.

- Although, many compounds have been shown to induce pathogenesis related proteins the infection resistance is not the same for all cultivars or pathogens. However, the lesion size for a given infection can be inversely correlated with the dose of the inducing chemical administered and, if application
15 of a given set of inducers at a concentration great enough to induce production of an array of pathogenesis related proteins, then the pathogen insult could be minimized to the extent that no signs of infection would be visible. That is to say, there would be no visible lesion formation.

- So then, the application of several compounds known for inducing or
20 eliciting the production of different classes of pathogenesis related enzymes simultaneously provides greater protection against infection, prevents or minimizes the rate of infection and, if administered after infection, halts the infection.

- Many compounds have been documented to induce the production of
25 ethylene in plants. Auxins, a class of plant hormones, have been shown to promote the production of the gas in plants at low concentrations, i.e., Auxins may be used to elicit pathogenesis related proteins for plant defense. Because Auxins have been well studied and auxin structure activity relationships have been identified, synthetic compounds with auxin like biological characteristics are
30 readily contrived. Naturally occurring Auxins typically have an indole core structure. The indole becomes biologically active with either auxin or anti-auxin

-4-

like activity with molecule functionality substitution. With the use of resonance structures the structural similarities are easily seen. Therefore, the use of benzothiadiazoles and their derivatives as biologically active materials may be actually mimicking Auxins. The relationship between ethylene and Auxins has
5 been well documented. The promotion of pathogenesis related proteins from exposure to ethylene is also well documented, e.g., the use of Auxins and other ethylene elicitors for the purpose of inducing an array of pathogenesis related proteins is now documented.

Ethylene is also documented to be expressed from injury to a plant. If one
10 were to compare pathogenesis related protein production from known elicitor applications then the difference in those proteins induced would be from either concentration applied, compound applied, or compounds application method. Because the amount of pathogenesis related enzymes expressed is roughly proportional to the amount of inducer applied and because lesion size is
15 proportional to the amount of pathogenesis related protein produced, the amount of an inducement elicitor applied should maximize the promotional properties of the inducement elicitor without causing harm. For salicylic acid an optimum concentration would be greater than 75mM.

Substituted phenyl compounds are usually defined as aromatic
20 substances that possess one or more functional groups. Such compounds are essential for the regulation of plant growth and development. Phenolics are plant growth inhibitors. Natural growth inhibitors (phenolics) are found in growing and dormant plants, fruits and seeds. These compounds are thought to work in concert with the phytohormones to regulate the growth and development of
25 organisms throughout their life cycle.

Some of the regulatory functions of these compounds include: signal producers for interactions with other organisms, growth regulation and structural integrity of each cell in the organism. Salicylic acid application has also been found to inhibit ethylene biosynthesis, inhibit germination, block wound
30 responses, interfere with membrane ion transport and absorption of roots, induce rapid membrane depolarization and collapse of the transmembrane

electrochemical potential, reduce transpiration in leaves and epidermal strips, reverse ABA induced stomatal closure, affect leaf abscission, and affect growth inhibition. These responses will almost certainly cause a decrease in the potential yield of a crop.

5 United States Patent No. 5,654,414 to Ryals, et al, states "... in order to achieve a desirable phenotype the chimeric gene may need to be expressed at levels of 1% of the total protein or higher. This may be the case for fungal resistance due to increased proteinase inhibitor expression. In cases where the energy expended to produce high levels of foreign protein may result in a
10 detriment to the plant whereas, expression of the gene only when desired, for instance when a fungal or insect infestation is imminent, would result in reduced drain on energy, and therefor yield.

However, because salicylic acid has a K_a of 1.05×10^{-3} it would not generally exist as a protonated acid in the xylem, phloem or cytoplasm; a
15 conjugate base or ester would probably exist in such an environment. The use of weak bases, specifically amines, for the neutralization of the weak acid would mimic the naturally occurring compounds as found in nature. High concentrations of phenolics have been found in the presence of amines during flowering.

20 Substituted phenyl compounds have long been known to inhibit the growth in plants. Nonetheless, some phenolic acids are known to induce the production of proteins that provide the SAR. The SAR acts as an immunity response that protects the plant against fungus, bacteria, and virus. This response is generally triggered from interactions between the plant and
25 pathogen, i.e., viral, fungal, and bacterial agents. The immune response is a systemic gene expression that requires phenolic accumulation. The SAR generally lasts weeks to months.

Because many pathogens attack the leaf surface of a plant, and, that the first barrier to the pathogen is typically the cuticle, and that cuticle consists of
30 fatty acid esters; the cuticle is soluble in alcohol or ketone. The use of hydroxyl, carbonyl, or ketal functional groups in combination with the chelated metal ions

-6-

may aid in the cation permeability through the cuticle to the plant cell. It has been found that the use of copper salts, either applied to the leaf or soil, when present in the cell at 10 to 100 times the amount necessary for normal growth and development, may control pathogens. Because of the efficacy of chelated copper the amount of copper salt necessary at an application to the soil or the plant itself to raise the copper content in tissue analysis is lowered. In copper deficient soils two kilogram/hectare of chelated copper when foliar applied has been shown to add enough copper ion into the plant tissue. An newfound method of foliar application of chelated copper at a concentration of 0.006M (equivalent to 0.25-0.5 kilogram/hectare) has been shown to raise the dry weight tissue analysis to levels of at least 10 to 60 times the amount necessary for normal growth and development; this new method is at least 2 to 10 times more effective than synthetic compounds from a foliar application. That is to say, if synthetic chelates were neutralized with the organic amine that the same efficiency might be found. It may be the osmotic potential of inorganic salts that sets up a directional gradient opposite of the intended effect that minimizes the actual absorption of the cation. So then, the use of the organic polyfunctional amines with the synthetic chelates may increase their efficacy.

Because plants are typically under disease pressure from a number of pathogens, and, the enzymes expressed from a given elicitor are somewhat pathogen specific, the application of a several elicitors offers greater protection. If the elicitors were then delivered with a melanin synthesis inhibitor then protection would be universal against fungi. So application of several compounds known for inducing or eliciting the production of different classes of pathogenesis related enzymes simultaneously should provide greater protection against infection, prevent or minimize the rate of infection, and if administered after infection, halt infection.

Accordingly, those skilled in the art had sought methods for obtaining the perceived benefits of disease inhibition by treatment with salicylic acid without causing resultant plant growth inhibition. Thus, there has been a long felt but

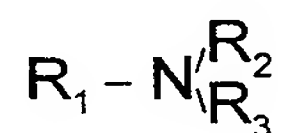
-7-

unfulfilled need for methods for achieving that end. The present invention solves that need.

Summary of the Invention

The present invention is directed to processes for improving plant resistance to disease without inhibiting plant growth. These processes are broadly directed at treating the plant with an ortho-substituted benzoic acid and nitrogen-containing compounds that is selected from the group consisting of the organic amines and compounds which will metabolize to an organic amine. These processes have been found to be further improved by simultaneously treating the plant with a chelated micronutrient metal. Finally, even more improvement has been observed where the plant is further treated with an ethylene inducing compound. Alternatively, it has been found that the benzoic acid and derivatives and nitrogen-containing compounds may be applied as an amine salts of the substituted benzoic acid. The acid, nitrogen containing compound, and optionally, chelated micronutrient metal and ethylene-inducing compounding, should be applied to the foliage of the plant as a solution flocced with a suitable carrier medium.

The nitrogen-containing compound is selected from the group consisting of the polyamines, tertiary amines, propylamines, and organic compounds which will metabolize to produce a propylamine. Preferred compounds are the organic amines having the structure



where R_1 , R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen and alkyls and substituted alkyls having not more than three carbon atoms, provided that R_1 , R_2 and R_3 are not all hydrogen. Most preferred are monoethanolamine, propylamines and dimethylaminopropylamine (DMAPA).

The preferred ortho-substituted benzoic acid is salicylic acid. The chelated micronutrient metal preferably is selected from the group consisting of

-8-

the alkaline earth metals, the transition metals and boron. The ethylene-inducing compound of choice is indole-3-butyric acid.

The method of the present invention has been found to increase the resistance to disease exhibited by a variety of crop plants. Most significantly, this increased resistance was achieved without the deleterious effects which condemned prior attempts to employ salicylic acid to prevent or minimize disease in plants. This result has been achieved by simultaneous application of a nitrogen-containing compound, most preferably a tertiary amine or propylamine in accord with the present invention. Accordingly, the methods of the present invention have solved the problems which previously plagued attempts to capitalize on the benefits associated with the use of salicylic acid.

Thus, the long felt, but unfulfilled need for improved methods for enhancing plant disease resistance without inhibiting plant growth have been met. These and other meritorious features and advantages of the present invention will be more fully appreciated for the following description and claims.

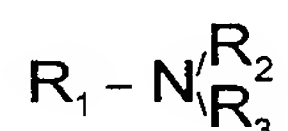
Detailed Description of the Preferred Embodiments

The present invention provides processes for improving plant resistance to disease without inhibiting plant growth. The processes of the present invention overcome the problems which plagued prior attempts to employ salicylic acid and related acids and salts. For example, while it has been known that treatment of plants with salicylic acid can inhibit disease, the treatments, unfortunately, have also resulted in a significant stunting, and even death, of the plants. It has been impossible to previously take advantage of the beneficial results of salicylic acid treatment. Surprisingly, Applicant has discovered that when treatment with the acid is combined with a nitrogen-containing compound, preferably selected amines, the deleterious effects have been overcome and plant resistance to disease have been improved without any adverse effect on growth.

The present invention is broadly directed to a process for improving plant resistance to disease without inhibiting plant growth. In its broadest aspect, the present invention is directed to the treatment of plants with an ortho-substituted

-9-

benzoic acid and a nitrogen-containing compound selected from the group consisting of the organic amines and compounds which will metabolize to an organic amine. The most preferred ortho-substituted benzoic acid is salicylic acid. The preferred nitrogen-containing compounds are selected from the group consisting of the polyamines, tertiary amines, propylamines and organic compounds which will metabolize to provide a propylamine. A particularly preferred group of amines are those having the structure



where R_1 , R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen and alkyls and substituted alkyls having not more than three carbon atoms, provided that R_1 , R_2 and R_3 are not all hydrogen. Particularly preferred are monoethanolamine, propylamines and DMAPA and mixtures thereof. Alternatively, it has been found that the acid and amine may be applied as the amine salt of the acid. Most preferred are the amine salts of salicylic acids.

Further methods have been achieved by including with the treatment a chelated micronutrient metal. Preferably these metals are selected from the group consisting of the alkaline earth metals, the transition metals and boron. Exemplary cations include copper, zinc and manganese.

Finally, it has been found that inclusion of an ethylene-inducing compound in the treatment may be beneficial. An example of such an ethylene-inducing compound is indole-3-butyric acid.

The ortho-substituted benzoic acid and nitrogen-containing compound, together with the optional chelated micronutrient metal and ethylene-inducing compound, should be applied to the foliage of the plant. Preferred treatment includes foliar application of the solution of those compounds in a suitable carrier medium. While the most preferred carrier medium is water, fertilizer solutions and any agriculturally acceptable medium may be employed. The ortho-substituted benzoic acid should be present in the treating solution in a concentration of not more than about 0.1M, preferably in the range of about

-10-

0.0001M to about 0.01M. However, the nitrogen-containing compound may be present in significantly higher percentage, up to about 25 percent-by-weight.

In order to facilitate a further understanding of the invention, the following examples primarily illustrate certain more specific details thereof.

5 **Example 1**

A solution of chelated metal ions, neutralized with sodium hydroxide, consisting of 1.5×10^{-2} M Cu (II), 1.5×10^{-2} M Zn (II), 1.8×10^{-2} M Mn (II), and a 3.8×10^{-3} M sodium salt of salicylic acid was applied to snap dragons. The plants were severely damaged by the application and death of some plants
10 followed. While at the same time a solution of chelated metal ions, neutralized with polyfunctionalamines, consisting of 1.5×10^{-2} M Cu (II), 1.5×10^{-2} M Zn (II), 1.8×10^{-2} M Mn (II), and 3.8×10^{-3} M amine of salicylic acid was applied to snapdragons without any visible damage occurring.

Example 2

15 Gerber Daisy infected with powdery mildew was treated with a solution in accord with the present invention in a university study. A concentrated solution containing 10 grams/liter salicylic acid, 20 grams/liter DMAPA and two percent each of copper (II), zinc (II) and manganese (II) was prepared. The metals were provided in a form of the oxides chelated with citric acid and monoethanolamine.
20 This concentrated solution was diluted 50 to 1 with water. The concentration of components in the following solution was about 0.0015M amine salt of salicylic acid, 0.006M each copper (II) and zinc(II) and 0.007M manganese (II). Commercial available Auxin (indole-3-butyric acid) was added as an ethylene-inducing compound. The solution so prepared, together with a control of water,
25 was applied to Gerber Daisy infected with powdery mildew. A single foliar application was found to prevent infection. Subsequent applications of the same solutions applied at two week intervals continued to prevent infection. All plants treated with salicylic acid/amine solution were found to be free of visible lesions at the end of the study. The control plants, treated only with foliar applied water,
30 all died.

Example 3

-11-

In a field test covering approximately 700 acres of watermelon infected with anthracnois was treated with a solution in accord with the present invention together with control. A concentrated solution was prepared in accord with the description in Example 2. A final solution for application was prepared 19 to 1
5 dilution with water. The concentration of components in the final solution is about 0.0038M amine salt of salicylic acid, 0.015M each copper (II) and zinc (II) and .018M manganese (II). Again, Auxin was added as an ethylene-inducing compound. A single treatment with the foregoing solution prevented further infection by anthracnois. After two weeks, a second treatment with an identical
10 solution was applied. All plants treated were free of further visible signs of disease. The controlled portion of the field, being treated only with foliar applied water, had no harvest. In fact, substantially all of the untreated plants died.

Example 4

Celery was successfully treated in accord with the present invention by a
15 similar solution. A final solution containing salicylic acid, an amine, chelated micronutrient metals and an ethylene-inducing compound was prepared in accord with that described in Example 3. That solution was foliar applied to celery in a Florida field. Three applications were made during the growing season. While no disease was present, an increase in biomass and size was
20 recorded at harvest. Control plants were treated with water. Using a sample of thirty treated and thirty controlled, biomass and size were recorded at harvest. Harvest mass of the controlled plants averaged 1.04kg per plant while those treated with the salicylic acid/amine solution averaged 1.32kg per plant. Height of the plants was also increased from an average of 61cm to 64cm. Thus,
25 treatment in accord with the present invention, far from stunting or inhibiting plant growth, resulted in a 5 percent increase size, together with a 27 percent increase in biomass.

Example 5

A series of test were performed by an independent laboratory to test salicylic acid/amine solutions against industry standards. The molar concentrations of components in solutions were as follows:

	Coppe r	Zinc	Mangenese	Salicylic Acid	Auxin
Solution 1	0.012	0.012	0.015	0.003	1.5×10^{-5}
Solution 2	0.0062	0.0061	0.0073	0.0015	7.5×10^{-6}
Solution 3	0.0031	0.003	0.0036	0.00075	3.8×10^{-6}

-13-

The solutions described above were applied both prior to (protective) and after (curative) inoculation with pathogens. The results are shown in the following table:

Treatment	Rate	<i>P. infestas</i> (late blight on tomatoes)	<i>P. oryzae</i> (rice blast on rice)	<i>P. recondita</i> (wheat rust on wheat)
Protective				
	Solution 1	100.0	90.0	80.0
	Solution 2	100.0	80.0	50.0
	Solution 3	100.0	0.0	0.0
	(ppm)			
Metalaxyl	30	92.7	-	-
Metalaxyl	10	60.0	-	-
Benomyl	100	-	100.0	-
Benomyl	40	-	40.0	-
Propiconazole	5	-	-	100.0
Propiconazole	1		-	36.7
Curative				
	Solution 1	80.0	43.0	0.0
	Solution 2	65.0	0.0	0.0
	Solution 3	36.7	0.0	0.0
	(ppm)			
Metalaxyl	30	81.7	-	-
Metalaxyl	10	48.3	-	-
Benomyl	100	-	100.0	-
Benomyl	40	-	35.0	-
Propiconazole	5	-	-	97.7
Propiconazole	1		-	43.3

Example 6

Corn was treated with a solution in accord with that prepared in Example 3 except that a 50:1 dilution with water was used. While disease was not present during the growing period, an increase in grain mass was recorded at harvest. Average yield per acre at harvest for controls (treated with water) was about 181.9 bushels per acre; average for the portion of the field treated in accord with the present invention was about 192.5 bushels per acre. In both cases nitrogen was applied at a rate of 100 pounds per acre.

Example 7

Red Oak Lettuce was successfully treated in accord with the present invention in an experiment conducted at the University of Queensland in Australia. A single spray at early seedling stage soon after planting prevented disease. Further, significant increases both diameter and height were observed. A treatment solution in accord with that described in Example 3 above was prepared and foliar applied to the plants at early seedling stage. Ten plants were treated with that solution while an additional ten plants were maintained as a control treated with water. Average plant diameter increased from 25.2cm to 27.7cm, an increase of about 10 percent. Similarly, average plant height increased from 14.8 to 16.7cm, an increase of about 13 percent. Thus, not only did treatment of growth in the present invention prevent disease, but surprisingly produced significant increase in yield.

Example 8

In another experiment using Red Oak Lettuce, yield was shown to be significantly improved. The application was designed and conducted in accord with that described in foregoing Example 7. Again, 10 plants were treated with a solution in accord with the present invention while 10 were maintained as controls treated with water. Tops and roots were collected, cleaned and weighed at harvest. Average top weight increased from 186.40gm to 226.67gm, an increase of 22 percent. Average root weight increased even more dramatically, from 59.80gm to 78.89gm, an increase of about 32 percent. Finally, total weight increase from 246.20gm to 305.56gm, an increase of 24

-15-

percent. Thus, not only did treatment in the present invention prevent disease, but surprisingly produced significant increase in yield.

Example 9

Another test using Green Oak lettuce was set up to measure disease and plant diameter. A single application of the solution described in Example 7 was made at the early seedling stage soon after transplanting. Fifteen plants were treated with that solution while fifteen more were maintained as controls treated with water. Diameter was determined by measuring the extremities of the outer leaves. Disease ratings was made by removing a plant from its whole and examining the roots. Ratings were graded on a visual scale from 1 to 5; of the roots graded 1 with no sign of infection while root showing severe pythium damage were rated 5. Treatment in accord with the present invention increased average plant diameter from 18.93cm to 23.07cm, an increase of 22 percent. An even more dramatic improvement was seen with respect to disease rating. The control averaged a 3.38 disease rating while that plants treated in accord with the present invention were dramatically decreased to an average of 2.00. Thus, a spectacular 59 percent improvement in disease rating was achieved with one foliar application.

Example 10

The example demonstrates the effect of salicylic acid in growth inhibition when used with other amines. The work was conducted at Texas A&M Crop Biotechnology Center, College Station, Texas.

Each seed was planted, five seeds per pot, and thinned to two per pot after germination. No fertilizer was employed. Foliar applications were made when the plants were 18 days old. The tops of the plants were then harvested two weeks later and dried in an oven. The following four blends were used for the foliar applications:

Blend 1		<u>Grams</u>
30	Water	460
	DMAPA	5
	Salicylic Acid	5

-16-

Blend 2 **Grams**

5	Water	500
	MEA	200
	Citric Acid	200
	Salicylic Acid	8
	DMAPA	17
	Formic Acid	10

Blend 3 **Grams**

10	Water	460
	Salicylic Acid	5
	DMAMP (80%)	4

Blend 4

Water Control

15

It was observed that the dry weight was not decreased by the use of a salicylic acid, the presence of the amine prevented growth inhibition. Indeed, it was noted that when using Blend 1 there was a slight increase in dry weight over that achieved with the control as by using blends 2 and 3 dry weight decreased insignificantly.

Example 11

The procedure of Example 6 was followed with the exception that no nitrogen was applied. The test plants were soybeans. It was found that the average unit per acre at harvest for the control (treated with water) was 44.6 bushels per acre while the average field per acre for plants treated with the solution used in Example 6 was 49.4 bushels per acre. It is to be noted that in both cases of Example 6 and in this Example, the solution was applied as a 2% solution.

-17-

The foregoing description of the invention has been directed in primary to a particular preferred embodiment in accord with the requirements of the Patent Statutes and for purposes of explanation and illustration. It will be apparent, however, to those skilled in the art that many modifications and changes in the specifically described methods may be made without departing from the true scope and spirit of the invention. For example, while salicylic acid and an amine are preferred, other organic acids and amines may be used. Further, the amine salts of salicylic acid may be employed. Also, while it is preferred to dilute the acid/amine salts in aqueous solutions, it may also be delivered in other agriculturally acceptable carriers. Further, while Applicant has attempted to explain the reasons for the unexpectedly improved results achieved, Applicant does not wish to be held to the theory proposed because that mechanism is not fully understood. Therefore, the invention is not restricted to the preferred embodiment described and illustrated but covers all modifications, which may fall within the scope of the following claims.

-18-

What is claimed is:

1. A process for improving plant resistance to disease without inhibiting plant growth, comprising treating the plant with:
 - an ortho-substituted benzoic acid;
 - 5 a nitrogen-containing compound selected from the group consisting of the polyamines, tertiary amines, propylamines, and organic compounds which will metabolize to produce a propylamine;
 - a chelated micronutrient metal selected from the group consisting of the alkaline earth metals, the transition metals and boron; and
 - 10 an ethylene-inducing compound.
2. The process of Claim 1 wherein the plant is treated with a solution having from about 0.0001M to about 0.01M salicylic acid and not more than about 25 percent-by-weight nitrogen-containing compound in a suitable carrier medium.
3. The process of Claim 2 wherein said said nitrogen-containing compound is selected from the group consisting of monoethanolamine, propylamines and dimethylaminopropylamine.
4. The process of Claim 3 wherein said ethylene-inducing compound is indole-3-butyric acid.
5. The process of Claim 1 wherein said treating comprises treating the foliage of the plant.

-19-

6. A process for improving plant resistance to disease without inhibiting plant growth, comprising:

treating a plant with salicylic acid and a nitrogen-containing compound selected from the group consisting of the polyamines, tertiary amines, propylamines and organic compounds which will metabolize to produce a propylamine.

7. The process of Claim 6 wherein said plant is further treated with a chelated micronutrient metal.

8. The process of Claim 7 wherein said metal is selected from the group consisting of the alkaline earth metals, the transition metals and boron.

10 9. The process of Claim 6 wherein the plant is further treated with an ethylene-inducing compound.

10. The process of Claim 6 wherein the plant is further treated with a chelated micronutrient metal and an ethylene-inducing compound.

11. The process of Claim 6 wherein the plant is treated with a solution having not more than about 0.05M salicylic acid and not more than about 25 percent-by-weight nitrogen-containing compound in a suitable carrier medium.

12. The process of Claim 11 wherein the concentration of acid in said solution is about 0.0001M to about 0.01M.

13. The process of Claim 6 wherein said salicylic acid and nitrogen-containing compound are applied as an amine salt of salicylic acid.

14. The process of Claim 6 wherein said treating comprises treating the foliage of the plant.

15. A process for improving plant resistance to disease without inhibiting plant growth, comprising:

treating the plant with an *ortho*-substituted benzoic acid and a nitrogen-containing compound selected from the group consisting of the organic amines and compounds which will metabolize to an organic amine;

16. The process of Claim 15 wherein said nitrogen-containing compound is an organic amine having the structure $R_1-N\begin{smallmatrix} R_2 \\ R_3 \end{smallmatrix}$ where R_1 , R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen and alkyls and substituted alkyls having not more than three carbon atoms, provided that R_1 , R_2 and R_3 are not all hydrogen.

17. The process of Claim 15 wherein said plant is further treated with a chelated micronutrient metal.

18. The process of Claim 17 wherein said metal is selected from the group consisting of the alkaline earth metals, the transition metals and boron.

19. The process of Claim 15 wherein the plant is further treated with an ethylene-inducing compound.

20. The process of Claim 15 wherein the plant is treated with a solution having not more than about 0.1M acid and not more than about 25 percent-by-weight nitrogen-containing compound in a suitable carrier medium.

21. The process of Claim 20 wherein the concentration of acid in said solution is about 0.0001M to about 0.01M.

-21-

22. The process of Claim 15 wherein said ortho-substituted benzoic acid and nitrogen-containing compound are applied as an amine salt of said ortho-substituted benzoic acid.

23. The process of Claim 15 wherein said treating comprises treating the foliage of the plant.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16365

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :AOIN 37/10, 43/36

US CL :504/138, 144, 324

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 504/138, 144, 324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RYALS et al. "Systemic Acquired Resistance" The Plant Cell 8:1809-1819. 1996 October Vol. pages 1809-1819, see entire document.	1-23
Y	LYON et al. "Novel Disease Control Compounds: The Potential to 'Immunize' Plants Against Infection." Plant Pathology 44:407-427. 1995, vol.44, pages 407-427, see especially page 412.	1-23
Y	PALVA et al. "Salicylic Acid Induced Resistance to Erwinia carotovora subsp. carotovora in Tobacco" Molecular Plant-Microbe Interactions. 1994 vol.7, no.3, pages 356-363, See entire document.	1-23



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 NOVEMBER 1999

Date of mailing of the international search report

23 DEC 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

S. MARIE CLARKE

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16365

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	SPELTZER et al. "Salicyclic Acid Induces Resistance to Alternaria solani in Hydroponically Grown Tomato" Phytopathology 89(9):722-777. 1999.	1-23
Y	GAFFNEY et al. "Requirement of Salicyclic Acid for the Induction of Systemic Acquired Resistance" Science, 06 August 1993, vol. 261, pages 154-756.	1-23
Y	NYVALL, Robert F. Field Crop Diseases Handbook. Westport, Connecticut: AVI Pub Co. 1979. pages. 1-7	1-23